Large-scale genome-wide analysis identifies genetic variants associated with cardiac structure and function

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BACKGROUND. Understanding the genetic architecture of cardiac structure and function may help to prevent and treat heart disease. This investigation sought to identify common genetic variations associated with inter-individual variability in cardiac structure and function.

METHODS. A GWAS meta-analysis of echocardiographic traits was performed, including 46,533 individuals from 30 studies (EchoGen consortium). The analysis included 16 traits of left ventricular (LV) structure, and systolic and diastolic function.

RESULTS. The discovery analysis included 21 cohorts for structural and systolic function traits (*n* = 32,212) and 17 cohorts for diastolic function traits (*n* = 21,852). Replication was performed in 5 cohorts (*n* = 14,321) and 6 cohorts (*n* = 16,308), respectively. Besides 5 previously reported loci, the combined meta-analysis identified 10 additional genome-wide significant SNPs: rs12541595 near *MTSS1* and rs10774625 in *ATXN2* for LV end-diastolic internal dimension; rs806322 near *KCNRG*, rs4765663 in *CACNA1C*, rs6702619 near *PALMD*, rs7127129 in *TMEM16A*, rs11207426 near *FGGY*, rs17608766 in *GOSR2*, and rs17696696 in *CFDP1* for aortic root diameter; and rs12440869 in *IQCH* for Doppler transmitral A-wave peak velocity. Findings were in part validated in other cohorts and in GWAS of related disease traits. The genetic loci showed associations with putative signaling pathways, and with gene expression in whole blood, monocytes, and myocardial tissue.

CONCLUSION. The additional genetic loci identified in this large meta-analysis of cardiac structure and function provide insights into the underlying genetic architecture of cardiac structure and warrant follow-up in future functional studies.

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Introduction

Heart failure (HF) is associated with substantial morbidity, mortality, and health care costs, and is increasing in prevalence with the aging of the global population (1). Hence, prevention and treatment of HF by identifying its genetic and environmental determinants is a public health priority. The identification of the

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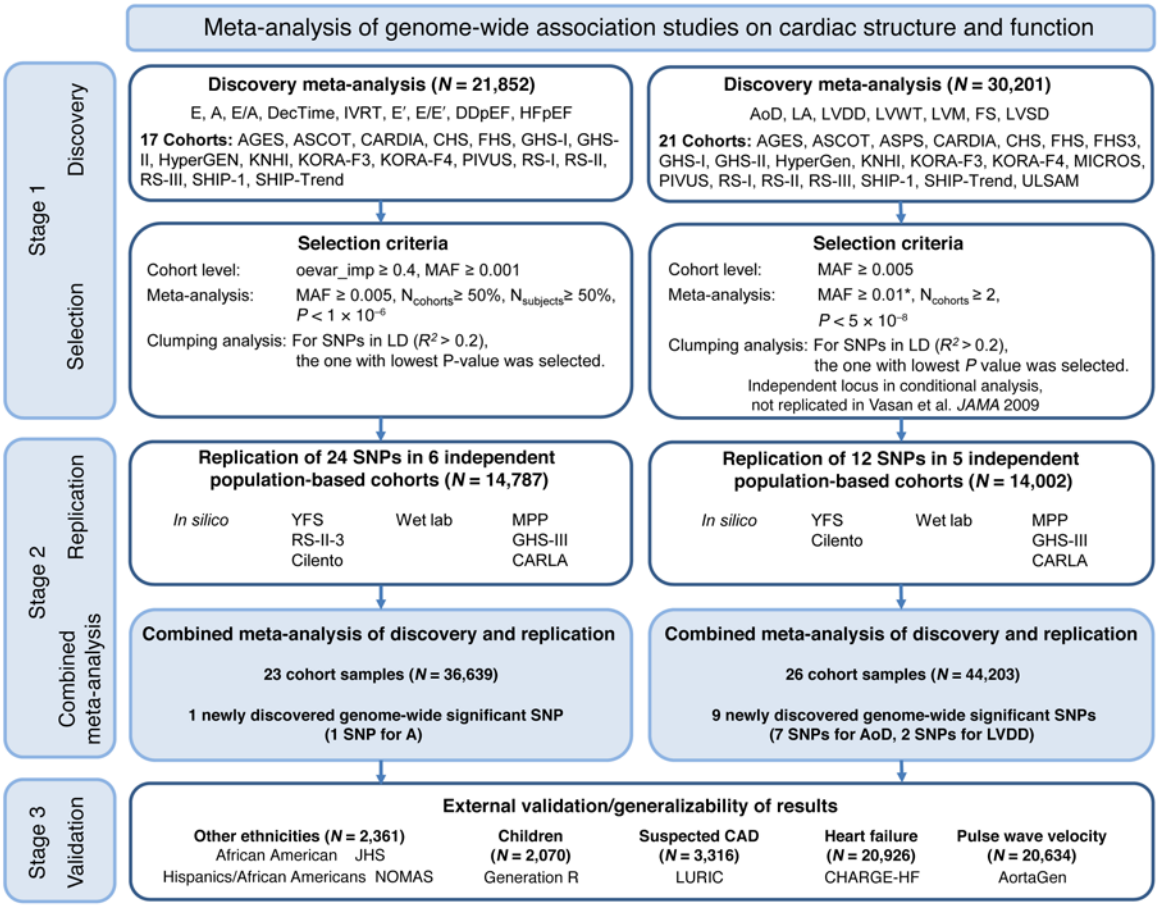

Figure 1. Flowchart of the analytical 3-stage approach. *For LV systolic dysfunction as binary trait, the selection criterion for the MAF was ≥0.03. Acronyms of cohorts are explained in the supplemental material. Mv-E (E), peak velocity of early diastolic transmitral inflow; Mv-A (A), peak velocity of transmitral inflow corresponding to atrial contraction; E/A, ratio of mitral E- and A-wave; DecTime, deceleration time of mitral E-wave; IVRT, isovolumetric relaxation time; E′, peak velocity of excursion of lateral mitral annulus in early diastolic phase; E/E′, ratio of E and E′; DDpEF, diastolic dysfunction with preserved ejection fraction; HFpEF, HF with preserved ejection fraction; LVM, LV mass; LVDD, LV diastolic dimension; LA, left atrium; FS, LV fractional shortening; LVSD, LV systolic dysfunction; MAF, minor allele frequency; $N_{cohorts}$, number of cohorts included in analysis; $N_{subjects}$, number of subjects investigated per phenotype; LD, linkage disequilibrium; CAD, coronary artery disease; oevar_imp., observed divided by expected variance for imputed allele dosage. Vasan et al. JAMA 2009 is ref. 2.

genetic architecture of HF may be facilitated by evaluating echocardiographic traits of left ventricular (LV) structure and function. These heritable, quantitative traits can antedate HF and are more amenable to genetic analysis than more "distal" heart disease traits (2). Initial studies that related common genetic variants to echocardiographic traits and incident HF (2–5) were limited by modest sample size, analysis of only a few echocardiographic phenotypes, or evaluation of "all HF," a phenotypically heterogeneous group (6–9).

We conducted a meta-analysis of genome-wide association studies (GWAS) on a comprehensive set of echocardiographic traits in carefully phenotyped individuals primarily of European ancestry within the EchoGen consortium (2) comprising 30 studies. We associated our identified genetic loci with echocardiographic traits in other ethnicities, in populations with related disease traits. Additionally, we further characterized loci by evaluating putative signaling pathways and examining their association with gene expression in whole blood, monocytes, and cardiac tissue.

Results

Cohort descriptions and the echocardiographic characteristics are presented in Supplemental Tables 1–5; supplemental material available online with this article; https://doi.org/10.1172/JCI84840DS1.

Individual study genomic inflation factors are shown in Supplemental Table 6. The meta-analytic genomic inflation factor (λ) was 1.09 or less for all traits evaluated. The genomic inflation factors for the traits with significant results (see below) were 1.09 (for aortic root diameter [AoD]) and 1.08 (for LV diastolic internal dimension [LVDD]). To address to what extent the genomic inflation might be due to unaccounted population stratification versus truly associated genetic markers, we applied the recently developed linkage disequilibrium (LD) score regression method to these two traits (10). The genomic inflation factor due to potential confounding bias reduced to 1.05 for AoD and to 1.03 for LVDD, suggesting that our meta-analytic approach accounted for population stratification reasonably well. Quantile-quantile (Q-Q) plots are shown in Supplemental Figures 1–16.

Table 1. Genetic loci associated with echocardiographic traits of LV structure and systolic function with genome-wide significance at $P < 5.0 \times 10^{-8}$ in the discovery dataset, replication results, and a meta-analysis combining discovery and replication data

SNP	Chr	Position	Nearest gene	Distance to nearest gene (kb)	SNP annotation	Effect/non-effect allele	EAF[A]	Discovery	Replication	Combined meta-analysis			
								P	*P*	Effect (SEM)	*P*	Heterogeneity I^2	Heterogeneity *P*
AoD (cm)													
rs806322[B,E]	**13**	**49739445**	***KCNRG***	**246.4**	**Unknown**	**A/G**	**0.61**	**6.70×10^{-15}**	**0.035**	**−0.021 (0.003)**	**2.22×10^{-15}**	**0**	**0.620**
rs6702619[C,D,E,F]	**1**	**99818834**	***PALMD***	**65.4**	**Unknown**	**G/T**	**0.50**	**6.89×10^{-15}**	**3.84×10^{-3}**	**0.021 (0.003)**	**$<1.10 \times 10^{-16}$**	**0**	**0.409**
rs10770612[G]	12	20121906	*PDE3A*	291.6	Unknown	A/G	0.80	3.20×10^{-12}	–	–	–	–	
rs17469907[G]	5	122556319	*CCDC100*	152.1	Unknown	A/G	0.72	1.02×10^{-11}	–	–	–	–	
rs1532292[F,G]	17	2044233	*SMG6*	0	Intron	T/G	0.61	1.29×10^{-11}	–	–	–	–	
rs10878359[G]	12	64690891	*HMGA2*	44.6	Unknown	T/C	0.36	1.62×10^{-11}	–	–	–	–	
rs17696696[H]	**16**	**73950853**	***CFDP1***	**0**	**Intron**	**G/T**	**0.59**	**1.96×10^{-9}**	**0.079**	**−0.016 (0.003)**	**2.68×10^{-10}**	**0**	**0.578**
rs7127129[E,F,H]	**11**	**69705561**	***TMEM16A***	**0**	**Intron**	**G/A**	**0.41**	**2.45×10^{-9}**	**0.303**	**−0.015 (0.003)**	**2.44×10^{-9}**	**0.20**	**0.292**
rs17608766[C,D,E,F,H]	**17**	**42368270**	***GOSR2***	**0**	**Intron**	**C/T**	**0.14**	**4.28×10^{-9}**	**0.020**	**0.0244 (0.0038)**	**2.25×10^{-10}**	**0.66**	**0.032**
rs2649	15	61673646	*USP3*	2.9	Untranslated-3′	T/C	0.13	1.01×10^{-8}	0.535	−0.021 (0.004)	5.37×10^{-8}	0.67	0.029
rs4765663	**12**	**2049021**	***CACNA1C***	**0**	**Intron**	**C/G**	**0.16**	**1.39×10^{-8}**	**0.068**	**−0.020 (0.003)**	**4.00×10^{-9}**	**0**	**0.925**
rs11207426[D]	**1**	**59458507**	***FGGY***	**76.8**	**Unknown**	**A/G**	**0.37**	**2.93×10^{-8}**	**0.021**	**0.017 (0.003)**	**2.76×10^{-9}**	**0**	**0.518**
LVDD (cm)													
rs11153730[G]	6	118774215	*SLC35F1*	28.7	Unknown	T/C	0.51	6.40×10^{-16}	–	–	–	–	
rs12541595	**8**	**125926540**	***MTSS1***	**116.7**	**Unknown**	**T/G**	**0.30**	**3.02×10^{-12}**	**4.03×10^{-3}**	**−0.023 (0.003)**	**1.65×10^{-13}**	**0**	**0.513**
rs10774625[D,H]	**12**	**110394602**	***ATXN2***	**0**	**Intron**	**G/A**	**0.50**	**1.90×10^{-8}**	**0.068**	**0.016 (0.003)**	**1.28×10^{-8}**	**0.67**	**0.011**
LVM (g)													
rs1454157	4	177595792	*SPCS3*	108.4	Unknown	C/T	0.73	4.41×10^{-9}	0.301	1.384 (0.260)	9.68×10^{-8}	0.52	0.066
FS (%)													
rs9470361	6	36731357	*CDKN1A*	23.1	Unknown	A/G	0.25	5.30×10^{-9}	0.523	0.169 (0.036)	2.87×10^{-6}	0.62	0.021

[A]From combined meta-analysis. [B]As a proxy for rs2762049, $R^2 = 1.0$, D′ = 1.0. [C]Locus found in discovery phase but not replicated in the previously published meta-analysis (2). [D]Located within enhancer histone marks in ENCODE (17). [E]Located within DNase-hypersensitive sites in ENCODE (17). [F]Locus colocalizes with DEPICT prioritized gene (Supplemental Table 15). [G]Known locus (2), not taken forward for replication. [H]Significantly associated with transcripts in *cis* (see text for details). Chr, chromosome; EAF, effect allele frequency; LVDD, LV diastolic internal dimension; AoD, diameter of the aortic root; FS, fractional shortening. Boldface indicates novel replicated findings. Effects are β coefficients, which represent the change in echocardiographic measure in the units shown in the subheads (i.e., cm, g, or %) per unit difference in effect allele dose.

Single nucleotide polymorphisms related to cardiac structure and function (stage 1). We applied a two-stage design proposed by Skol et al. (11), including an additional stage for assessing the generalizability of the find, with details on samples and single nucleotide polymorphisms (SNPs) for each stage given in Figure 1. The meta-analysis of LV cardiac structure and systolic function traits included data from 21 cohorts with up to 30,201 individuals. For LV diastolic function, data were available from 17 cohorts with up to 21,852 individuals. We identified genome-wide significant associations (all $P < 5 \times 10^{-8}$) of: 1 locus with LV mass (LVM), 3 with LVDD, 12 with AoD, 1 with LV fractional shortening (LVFS). Additionally, the following associations were observed at a higher *P* value threshold (all $P < 1 \times 10^{-6}$): 2 with the peak velocity of the transmitral E-wave (Mv-E), 5 with the peak velocity of the transmitral A-wave (Mv-A), 5 with the ratio of Mv-E to Mv-A (E/A), 2 with deceleration time of Mv-E (DecTime), 4 with isovolumetric relaxation time (IVRT), 1 with the peak velocity of the excursion of the lateral mitral annulus in the early diastolic phase (E′), 3 with the ratio of Mv-E to E′ (E/E′), 1 with asymptomatic LV diastolic dysfunction with preserved ejection fraction (DDpEF), and 2 with HF with preserved ejection fraction (HFpEF). Using pre-defined selection criteria (Figure 1) and excluding known loci from our previous report (2), 12 SNPs for traits of cardiac structure and LV systolic function (Table 1) and 24 SNPs for traits of LV diastolic function (Table 2) were considered for additional analysis detailed in stage 2 below. Full results for all SNPs with $P < 1 \times 10^{-4}$ are shown in Supplemental Table 7.

Replication and combined meta-analysis (stage 2). SNPs taken forward for stage 2 replication were analyzed in 5 cohorts (n = 14,002; 2 with in silico GWAS data, 3 with de novo genotyping) for cardiac structure and LV systolic function; and in 6 cohorts (n = 14,787; 3 with in silico GWAS data, 3 with de novo genotyping) for LV diastolic function (Figure 1). A final combined meta-analysis of discovery and replication data from overall 30 cohort samples included 44,203 individuals with data on cardiac structure and systolic function, and 36,639 individuals with data on LV diastolic function. The investigation revealed 10 SNPs with genome-wide significance: rs10774625 and rs12541595 for LVDD; rs806322, rs4765663, rs6702619, rs7127129, rs11207426, rs17608766, and rs17696696 for AoD; and rs12440869 for Mv-A (Tables 1 and 2). Manhattan plots for these 3 traits are presented in Figure 2. Forest plots for the most significantly associated SNPs for AoD (rs6702619), LVDD (rs12541595), and Mv-A (rs12440869) with the corresponding regional plots including functional annotation are presented in Figures 3, 4, and 5. The plots for the other genome-wide significant loci are shown in Supplemental Figures 17 and 18. Funnel plots for the significantly associated SNPs are shown in Supplemental Figure 19. All known and novel loci combined explained 1.7%, 0.5%, and 0.2% of the phenotypic variance in AoD, LVDD, and Mv-A, respectively, in a combined analysis of 3 of the larger cohorts.

Findings in children, other ethnicities, and related cardiovascular phenotypes (stage 3). In stage 3, the genome-wide significant SNPs were investigated for generalizability of the observed associations; small sample sizes of available cohorts partly limited the statistical power to replicate findings. In this exploratory analysis, we only found one weak association with AoD in white children of European ancestry in the Generation R study (12), and none in Hispanics (Northern Manhattan Study [NOMAS] study) or African Americans (Jackson Heart Study [JHS] and NOMAS study; Supplemental Table 8). When evaluating associations of the newly discovered SNPs with related disease traits, rs17696696, which was found to be associated with AoD, was also associated with pulse wave velocity in the AortaGen consortium (Supplemental Table 9 and ref. 13). There were no statistically significant associations with incident HF or mortality in HF patients of the CHARGE-Heart Failure (CHARGE-HF) consortium (Supplemental Table 10), or with all-cause mortality, HF, or cardiovascular mortality in the Ludwigshafen Risk and Cardiovascular Health (LURIC) cohort of patients with suspected coronary artery disease (CAD) (Supplemental Table 11). In the CARDIOGRAMplusC4D consortium data, rs17696696, rs17608766, and rs10774625 were significantly associated with CAD; rs10774625 was also strongly associated with the narrower phenotype myocardial infarction (MI; $P = 5.09 \times 10^{-11}$, Supplemental Table 12).

Biological pathways related to echocardiographic traits. In pathway analysis, the observed genetic variants were significantly enriched for canonical pathways that might be involved in the biological regulation of echocardiographic traits: protein kinase A signaling ($P = 5.8 \times 10^{-6}$), death receptor signaling ($P = 6.9 \times 10^{-5}$), the Wnt/Ca^{2+} pathway ($P = 2.2 \times 10^{-4}$), and P2Y purigenic receptor signaling ($P = 4.1 \times 10^{-4}$, Supplemental Tables 13 and 14, Supplemental Figure 20, and refs. 14–16).

When investigating the potential regulatory effect of the top loci using Encyclopedia of DNA Elements (ENCODE) data (17), 4 SNPs (rs10774625, rs6702619, rs17608766, and rs11207426) were located within enhancer histone marks and 4 (rs806322, rs6702619, rs7127129, and rs17608766) within DNase-hypersensitive sites. The literature search tool Snipper revealed no additional information, and no significant direct or indirect protein-protein interactions were found between loci using DAPPLE software (18). No significantly reconstituted gene sets were identified by the DEPICT tool (ref. 19 and Supplemental Table 15). DEPICT prioritized (false discovery rate [FDR] <0.05) 10 genes across associated ($P < 1 \times 10^{-5}$) loci, including 4 colocalizing with genome-wide significant loci (Tables 1 and 2, and Supplemental Table 15).

Analyses of expression quantitative trait loci and gene expression in whole blood, monocytes, and myocardial tissue. Our data showed 4 SNPs that were significantly associated with *cis* transcripts in both datasets (whole blood and monocytes, Supplemental Table 16): rs10774625 with SH2B adaptor protein 3 (*SH2B3*, $P = 8.15 \times 10^{-20}$ and $P = 1.83 \times 10^{-4}$), rs17696696 with craniofacial development protein 1 (*CFDP1*, $P = 6.21 \times 10^{-11}$ and $P = 7.59 \times 10^{-5}$), rs7127129 with Fas-associated death domain–containing protein (*FADD*, $P = 1.61 \times 10^{-37}$ and $P = 2.71 \times 10^{-4}$), and rs1532292 with serine racemase (*SRR*, $P = 3.40 \times 10^{-20}$ and $P = 4.63 \times 10^{-10}$).

We also examined the associations of our top loci with gene expression in human LV tissue provided by the Myocardial Applied Genomics Network consortium (MAGNet consortium; unpublished data). Two SNPs were significantly associated with LV gene expression: rs12541595 showed *cis*-association with metastasis suppressor 1 (*MTSS1*, $P = 1.25 \times 10^{-19}$), with the effect allele T associated with lower *MTSS1* expression; rs1532292 showed again a *cis*-association with *SRR* ($P = 2.62 \times 10^{-4}$), with the effect allele T

Table 2. Genetic loci associated with echocardiographic traits of diastolic function with $P < 1.0 \times 10^{-6}$ in the discovery dataset, replication results, and a meta-analysis combining discovery and replication data

SNP	Chr	Position	Nearest gene	Distance to nearest gene (kb)	SNP annotation	Effect/non-effect allele	EAF[A]	Discovery	Replication	Combined meta-analysis			
								P	*P*	Effect (SEM)	*P*	Heterogeneity I^2	Heterogeneity *P*
Mv-E													
rs4690879	4	158215162	*GLRB*	1.6	Near-gene-5′	T/C	0.81	6.45×10^{-7}	0.550	0.639 (0.156)	4.45×10^{-5}	0.64	0.016
rs12564392	1	210842152	*ATF3*	0	Intron	A/C	0.02	6.41×10^{-7}	0.162	−0.994 (0.389)	0.011	0.78	$4,67 \times 10^{-4}$
Mv-A													
rs1039692[B]	6	54738825	*FAM83B*	80.7	Unknown	G/A	0.06	8.14×10^{-6}	0.258	−0.596 (0.237)	0.012	0.85	2.72×10^{-5}
rs11589479	1	153299932	*ADAM15*	0	Coding-synonym	A/G	0.17	6.18×10^{-8}	0.185	0.549 (0.133)	3.88×10^{-5}	0.72	3.54×10^{-3}
rs12440869[C]	**15**	**65398005**	***IQCH***	**0**	**Intron**	**T/A**	**0.26**	$\mathbf{1.90 \times 10^{-7}}$	$\mathbf{9.04 \times 10^{-3}}$	**−0.726 (0.128)**	$\mathbf{1.31 \times 10^{-8}}$	**0.54**	**0.054**
rs1447563	2	46490868	*EPAS1*	23.5	Unknown	C/A	0.47	5.13×10^{-7}	0.046	−0.586 (0.114)	2.76×10^{-7}	0.18	0.299
rs6905862	6	31235581	*TCF19*	0	Intron	A/G	0.42	2.90×10^{-7}	0.958	0.455 (0.120)	1.46×10^{-4}	0.65	0.015
E/A													
rs6791049	3	59728311	*FHIT*	0	Intron	T/C	0.06	1.14×10^{-7}	0.871	−0.019 (0.005)	1.05×10^{-4}	0.68	8.74×10^{-3}
rs1839005	14	41236693	*LRFN5*	0	Intron	C/A	0.21	1.65×10^{-7}	0.858	0.011 (0.003)	8.47×10^{-5}	0.62	0.021
rs7904368[B]	10	16898593	*RSU1*	0	Intron	T/C	0.79	8.31×10^{-7}	0.397	0.012 (0.003)	8.92×10^{-6}	0.62	0.022
rs12534994	7	46627074	*TNS3*	654.2	Unknown	C/T	0.20	6.94×10^{-7}	0.862	−0.010 (0.003)	2.11×10^{-4}	0.66	0.011
rs1891293	10	104991787	*INA*	35.1	Unknown	A/G	0.55	2.01×10^{-7}	0.947	−0.009 (0.002)	3.76×10^{-5}	0.59	0.032
DecTime													
rs1455795	8	76086655	*CRISPLD1*	0	Intron	A/G	0.10	2.82×10^{-7}	0.473	0.003 (0.001)	1.02×10^{-5}	0.5	0.091
IVRT													
rs4961252	8	142174126	*DENND3*	33.8	Unknown	A/G	0.62	4.31×10^{-7}	0.013	−0.002 (0.0004)	5.12×10^{-8}	0	0.474
rs6860194	5	68127986	*SLC30A5*	297.6	Unknown	G/C	0.24	1.44×10^{-7}	0.875	−0.002 (0.0004)	1.94×10^{-6}	0.46	0.115
rs9261387	6	30169340	*TRIM31*	9.3	Unknown	C/T	0.92	3.57×10^{-7}	0.347	0.003 (0.001)	6.97×10^{-7}	0	0.514
rs17868167[B]	2	51616109	*NRXN1*	507	Unknown	A/C	0.03	1.26×10^{-6}	0.785	0.004 (0.001)	6.49×10^{-6}	0.49	0.095
rs7729095	5	94414226	*MCTP1*	0	Intron	G/C	0.80	3.18×10^{-7}	0.795	−0.002 (0.0004)	9.14×10^{-6}	0.49	0.100
E′													
rs10484775	6	150967081	*PLEKHG1*	0	Intron	T/A	0.50	3.66×10^{-7}	0.897	0.055 (0.023)	0.014	0.81	3.61×10^{-4}
E/E′													
rs7139872	13	19975134	*CRYL1*	0	Intron	G/A	0.99	3.28×10^{-7}	0.724	−0.768 (0.184)	3.13×10^{-5}	0.61	0.037
rs1939680	11	115237927	*CADM1*	357.6	Unknown	C/G	0.55	6.30×10^{-7}	0.522	−0.043 (0.024)	0.068	0.83	9.95×10^{-5}
rs12068977	1	69455985	*LRRC7*	542.5	Unknown	C/G	0.01	7.79×10^{-7}	0.211	0.038 (0.069)	0.585	0.87	4.12×10^{-6}
DDpEF													
rs136772	22	48186471	*FLJ44385*	141.9	Unknown	A/G	0.90	7.44×10^{-7}	0.686	0.001 (0.010)	0.904	0.89	7.43×10^{-6}
HFpEF													
rs12304309	12	52493567	*CALCOCO1*	86.1	Unknown	C/T	0.98	2.34×10^{-7}	0.348	−0.037 (0.029)	0.207	0.90	1.60×10^{-6}

[A]From combined meta-analysis. [B]Proxy SNP for lead SNP of the locus. [C]Significantly associated with transcripts in *cis* (see text for details). Chr, chromosome; EAF, effect allele frequency; Mv-E, peak velocity of the early diastolic transmitral inflow; Mv-A, peak velocity of the transmitral inflow corresponding to atrial contraction; E/A, ratio of the mitral E- and A-wave; DecTime, deceleration time of the mitral E-wave; IVRT, isovolumetric relaxation time; E′, peak velocity of the excursion of the lateral mitral annulus in the early diastolic phase; E/E′, ratio of E and E′; DDpEF, diastolic dysfunction with preserved EF; HFpEF, HF with preserved EF. Boldface indicates novel replicated findings. Effects are β coefficients, which represent the change in echocardiographic measure in the units shown in the subheads (i.e., cm, g, or %) per unit difference in effect allele dose.

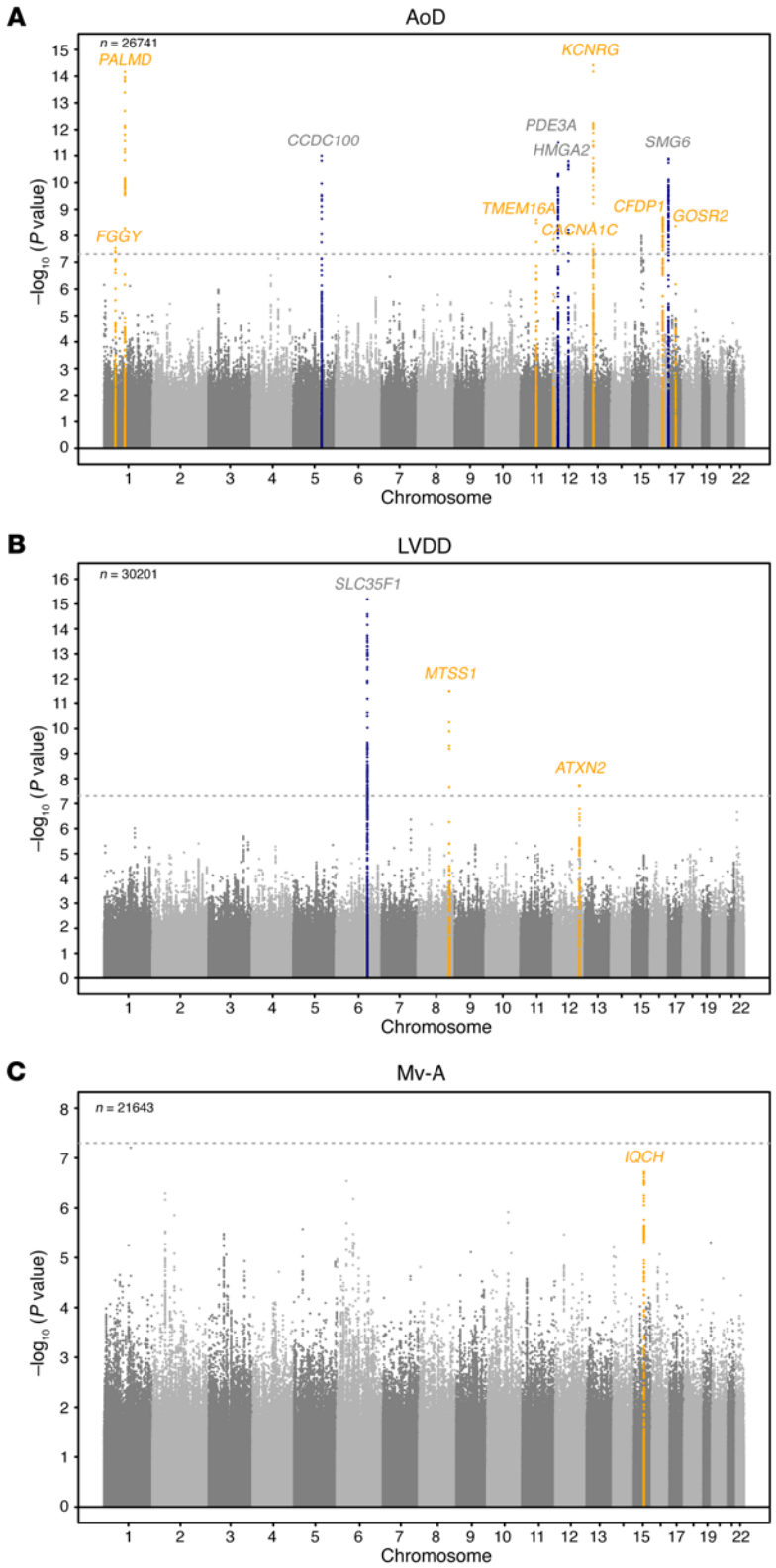

Figure 2. Manhattan plots of echocardiographic phenotypes with genome-wide findings in the joint analysis of discovery and replication cohorts. The plots show the SNP-wise log *P* values against their genomic position for (**A**) aortic root diameter (AoD), (**B**) LV diastolic dimension (LVDD), and (**C**) peak velocity of the transmitral inflow corresponding to the atrial contraction (Mv-A). The genome-wide significant loci and the gene nearest to the top SNP are highlighted in yellow if they were discovered in the present analysis and in blue if they had been identified in the earlier analysis. The horizontal gray lines indicate the significance threshold of $P = 5 \times 10^{-8}$. *P* values were obtained by calculating Wald test statistics. The number of samples (*n*) per trait is reported in the upper left corner of each panel.

associated with lower *SRR* expression. Both expression quantitative trait locus (eQTL) associations from the LV tissue were also supported by the GTEx database (http://gtexportal.org/home/). The association with *SRR* expression for rs1532292 had the same direction of effect in different tissues, with the T allele generally associated with lower gene expression levels, e.g., in the aorta and in blood cells. Additionally, the following eQTLs with genes from the reference sequence database (RefSeq; https://www.ncbi.nlm.nih.gov/refseq/) in the aorta or heart tissue were found for the replicated SNPs in the GTEx database: rs17696696 (*BCAR1*), rs12541595 (*LINC00964*), and rs11153730 (*SSXP10*). Detailed GTEx results are given in Supplemental Table 17.

Discussion

In the present investigation, we identified 7 genetic loci associated with aortic root size and confirmed the associations of 4 other loci previously reported (2). These 11 variants explained 1.7% of the inter-individual variation in aortic root size (Supplemental Table 18). However, use of genome-wide complex trait analysis (GCTA) software in one of the larger cohorts (Study of Health in Pomerania [SHIP]) as an illustrative example demonstrated that common genetic variation explains about 30% of the variation in AoD (Supplemental Table 19), underscoring the potential for more, as-yet-undiscovered, loci. Additionally, we observed three genetic loci that were associated with LV diastolic dimensions (including one previously reported; see below) and one locus that was associated with the transmitral A-wave velocity.

Among the SNPs identified in our study as being associated with LVDD, one was rs12541595 close to *MTSS1*, which interacts with cytoplasmic actin near the cell surface and modulates intercellular connections in the kidney and metastatic potential in tumors (20, 21). When investigating our top loci for *cis*-associations with gene expression in human LV myocardial tissue (MAGNet consortium, unpublished data) and the GTEx database, rs12541595 showed a significant association with *MTSS1* expression, with the LVDD-lowering allele (T) associated with lower *MTSS1* expression in this tissue (Supplemental Table 9). We speculate that a reduction in *MTSS1* may promote favorable LV remodeling, perhaps by affecting cell junctions. The other novel variant associated with LVDD, rs10774625, was associated with expression of *SH2B3* in eQTL analysis and lies in *ATXN2* (ataxin 2),

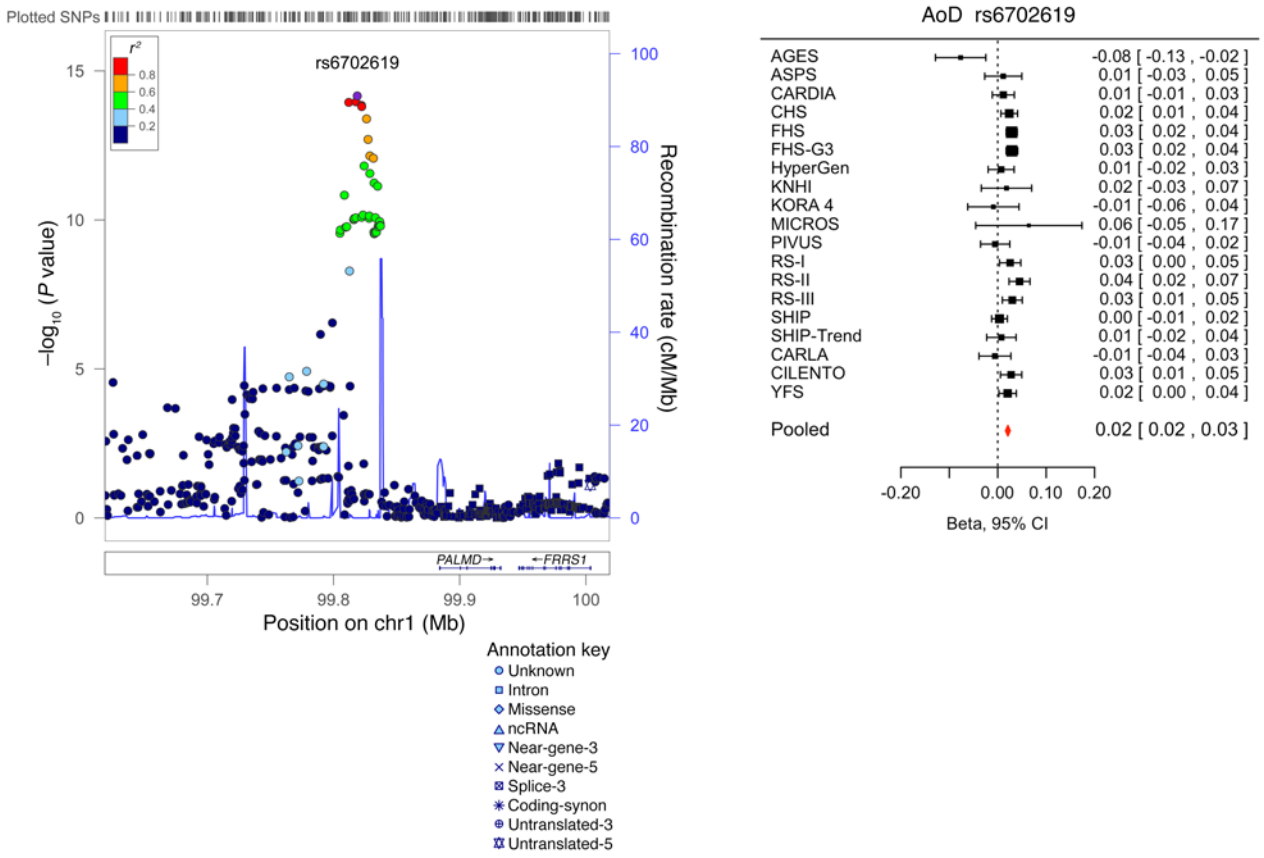

Figure 3. Forest plot for the meta-analysis of the association between rs6702619 and AoD, with the corresponding regional plot including functional annotation. *P* values were obtained by calculating Wald test statistics using a sample size of *n* = 26,741. Total sample size in the forest plot is *n* = 30,704.

which is adjacent to *SH2B3*, previously associated with retinal venular diameter, CAD, and arterial hypertension in separate reports (22–26). For LVDD, we also replicated the previously identified *SLC35F1* locus (soluble transporter membrane protein) adjacent to the phospholamban (*PLN*) locus (protein inhibiting cardiac muscle sarcoplasmic reticulum Ca^{++}-ATPase) (2).

Three loci associated with AoD have been linked previously to blood pressure as well as MI (*GOSR2*, Golgi SNAP receptor complex member 2; refs. 24, 27), blood pressure response to treatment (*CACNA1C*, calcium channel, voltage-dependent, L type, alpha 1C subunit; ref. 28), and carotid intimal-medial thickness, as well as with CAD (*CFDP1*; refs. 29, 30). The other novel AoD-associated genetic loci were in or close to *PALMD* (palmdelphin, a paralemmin-related cytosolic protein; ref. 31), *KCNRG* (soluble protein with regulatory function in voltage-gated potassium channels; ref. 32), *FGGY* (carbohydrate kinase domain–containing protein, phosphorylates carbohydrates; ref. 33), and in *TMEM16A* (transmembrane member 16A, protein involved in transepithelial anion transport and smooth muscle contraction; ref. 34). We also replicated in our discovery sample 4 loci associated with aortic diameter from our previous report (2): *SMG6* (Smg-6 homolog, nonsense-mediated mRNA decay factor), *CCDC100* (centrosomal protein 120kDa), *HMGA2* (high-mobility group AT-hook 2), and *PDE3A* (phosphodiesterase 3A, cGMP-inhibited). The effect allele of rs1532292 was associated with lower *SRR* expression in human LV myocardial tissue (unpublished data from the MAGNet consortium; GTEx database, see Supplemental Table 9).

One of the SNPs associated with AoD in our meta-analysis was also associated with AoD in children in the Generation R Study. Additionally, one SNP was associated with pulse wave velocity. Two SNPs associated with AoD and one SNP associated with LVDD were also significantly associated with CAD, the LVDD SNP also with MI in the CARDIOGRAMplusC4D consortium. These associations strengthen the evidence of involvement of these loci in echocardiographic traits. However, given the sample sizes of cohorts with different ethnicities as well as the SNP allele frequencies, and taking the effect sizes into account, the power was not more than 35% to reveal a statistically significant association of select SNPs with traits in "look-up" exercises. Therefore, some of the null results for the assessment of the generalizability of observed associations to non-European samples should be interpreted with care.

Pathway analysis suggested enrichment of the Wnt/Ca^{2+} canonical pathway among the genetic variants associated with echocardiographic traits. These observations are consistent with the known effects of this pathway on myocardial biology (35). The Wnt/Ca^{2+} pathway connects to the nuclear factor of activated T cells (NFAT) transcription factor (14, 15) and gene expression via calcineurin. Interestingly, both calcineurin and its target NFAT are involved in cardiac hypertrophy (16).

The association of our findings with expression data from human blood revealed 4 genes with potential functional signif-

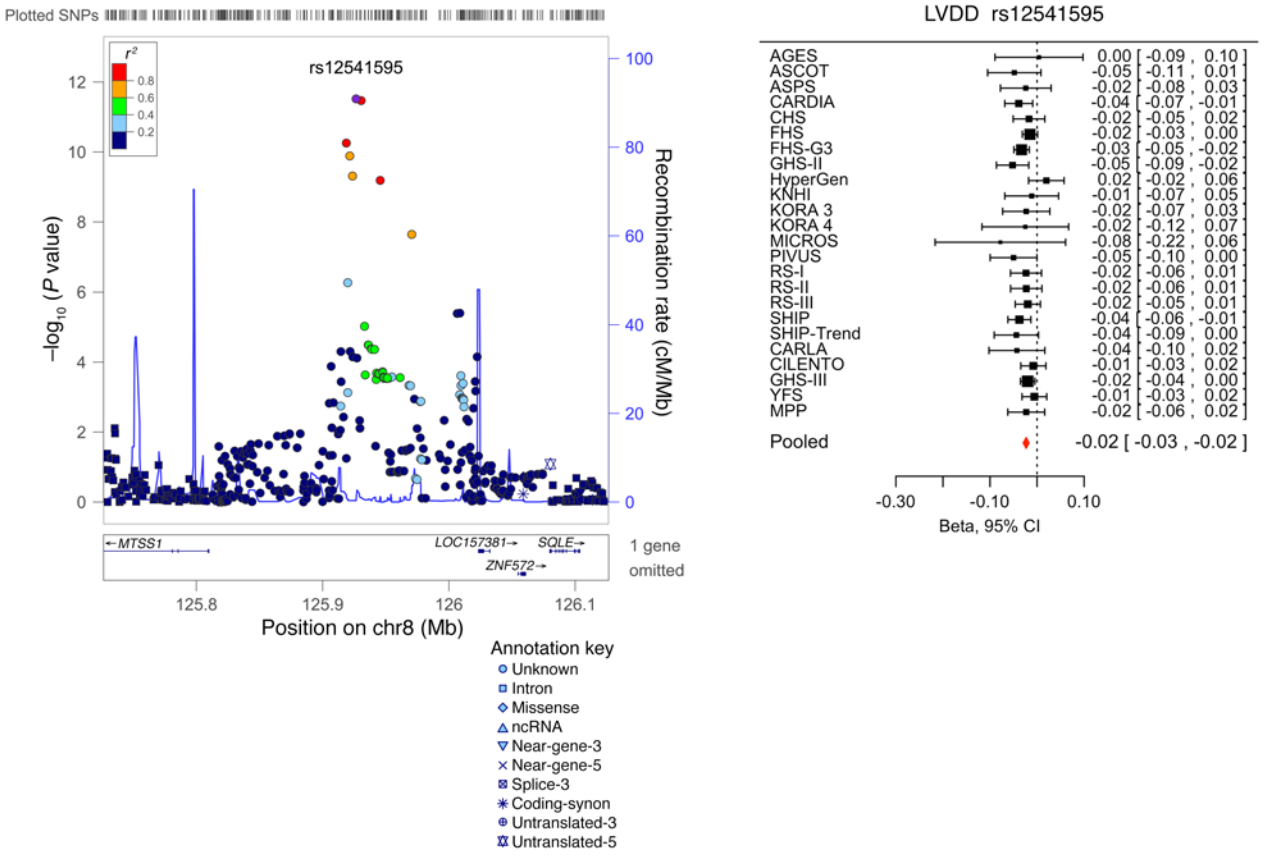

Figure 4. Forest plot for the meta-analysis of the association between rs12541595 and LVDD, with the corresponding regional plot including functional annotation. *P* values were obtained by calculating Wald test statistics using a sample size of n = 30,201. Total sample size in the forest plot is n = 43,623.

icance (Supplemental Table 8). Of these, rs7127129 is located within *TMEM16A*, but its eQTL *FADD* has been shown to be associated with myocardial ischemia/reperfusion injury in an HF mouse model (36).

Our study is strengthened by the large sample size, the use of standardized echocardiographic techniques with adequate quality, and a harmonization of phenotypic data. Nonetheless, several limitations must be acknowledged. We did not observe any association of common genetic variants with the other echocardiographic measurements studied, e.g., LA size, LV wall thickness (LVWT), LVM, LV systolic dysfunction (LVSD), and most measures of LV diastolic function, with the exception of the transmitral A-wave velocity. In particular, we did not find any statistically significant associations for HFpEF, although we included only carefully phenotyped individuals in our study to reduce the phenotypic heterogeneity (37). The lack of association of select echocardiographic traits with common genetic variation is intriguing. It is likely that heterogeneity in both phenotypic assessment and study design and modest statistical power may have limited our ability to detect modest genetic associations, and associations with rare variants could not be assessed by design. A proportion of the intra-individual variability of functional traits might have been influenced by physiological factors (e.g., posture, state of hydration, heart rate, or medication use) (38). In this context, it should be noted that some echocardiographic measures may be imprecise, e.g., analysis of tissue Doppler imaging (TDI) of the mitral annulus would likely have further improved diagnosis and classification of LV diastolic dysfunction in our study if this method had been available in more cohorts. Likewise, as noted above, several of the LV diastolic filling measures are notoriously susceptible to variation in ventricular loading conditions (38). The genetic variants identified in our study have small effect sizes and explain a relatively small percentage of the variance in the echocardiographic phenotypes. Larger studies with more detailed reference panels, as well as more detailed functional studies and studies into the interactions of the variants found with factors such as hypertension, will likely shed further light on the molecular mechanisms underlying these complex traits. Furthermore, alterations of the transmitral A wave velocity are challenging to interpret alone, without consideration of other measures of LV diastolic function and filling patterns. The transmitral A wave velocity reflects the late diastolic phase of the LV filling, i.e., the phase of atrial contraction. Thus, in theory this single measure provides important information about active atrial function. Yet in practice, this measure changes variably and in a complex manner with the progression of LV diastolic dysfunction: Increasing impaired ventricular relaxation is at first accompanied by a decrease in E-wave with a compensatory increase in A-wave, resulting in a "relaxation abnormality" pattern; it results in the further, continuous decrease in A-wave velocity, reflecting a progressive deterioration of the contractility of the left atrium, and also changes in LV compliance (39, 40). These pathophysiological considerations underline the importance of the active contraction

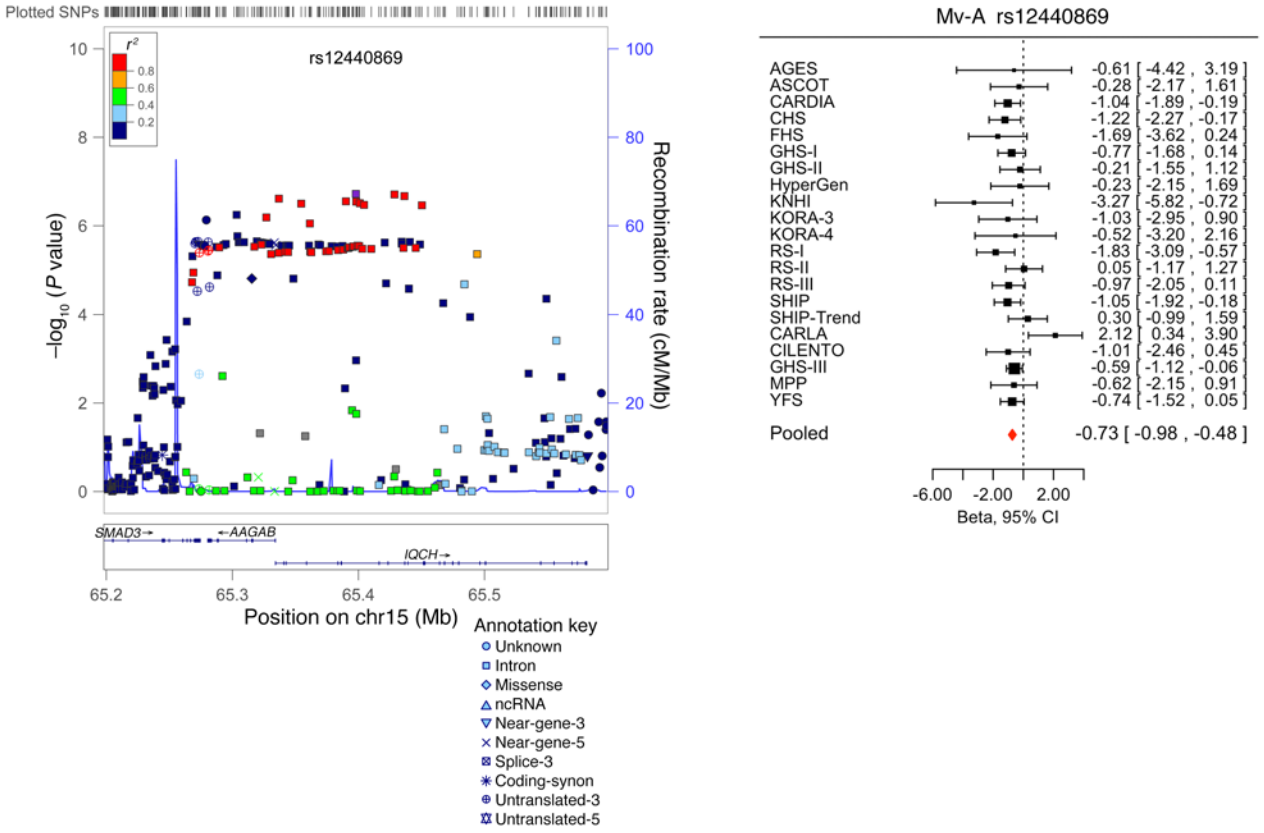

Figure 5. Forest plot for the meta-analysis of the association between rs12440869 and Mv-A, with the corresponding regional plot including functional annotation. *P* values were obtained by calculating Wald test statistics using a sample size of n = 21,156. Total sample size in the forest plot is n = 36,430.

of the left atrium. Last, we did not directly assess the functional significance of all the associated SNPs or perform mechanistic studies, other than for the *MTSS1* locus associated with LVDD (unpublished data from the MAGNet consortium).

To conclude, we report the largest genetic association study to our knowledge of a comprehensive set of LV echocardiographic traits. The large number of interesting genetic loci identified for AoD and LV diastolic dimensions, and the biological pathways enriched within our association results provide new insights into the biology of cardiac remodeling. Additional studies are warranted to further evaluate experimentally the functional significance of the reported genetic variants and loci.

Methods

EchoGen consortium

The EchoGen consortium was initiated in 2007 and has grown to a consortium of 30 studies with population-based and hospital-based cohorts primarily of European ancestry, and additionally including two cohorts of African American and one of Hispanic individuals. For the present investigation, we applied harmonized phenotype definitions, covariate selection, and genotyping protocols and the same statistical analysis plan across all cohorts. For traits of cardiac structure and systolic function, individuals with a history of MI, clinical diagnoses of HF, or valve disease were excluded if this information was known or recorded during the echocardiographic examination. For analysis of LV diastolic dysfunction, we excluded individuals with reduced ejection fraction (EF) (defined as <50%, LVFS <29% or poor/impaired LV systolic function by visual estimation).

Strategy for analysis

For the identification of genetic variants associated with cardiac structure and function, we followed a 3-stage analysis plan (Figure 1). First, a discovery meta-analysis of up to 21 population- and hospital-based GWAS was performed (stage 1). Second, replication of the findings from stage 1 was performed in up to 6 independent cohort studies (3 with in silico data and 3 with de novo genotyping), and a combined meta-analysis of discovery and replication data was carried out (stage 2). In stage 3, SNPs that were genome-wide significant in the combined meta-analysis were investigated for the generalizability of the observed associations in a cohort of white children of European ancestry (the Generation R study), in two cohorts of other ethnicities (Hispanic in the NOMAS Study and African American in the JHS and in the NOMAS study), and in associations with related disease traits (data from the AortaGen and CHARGE-HF consortia, and the LURIC study).

Echocardiographic methods

Detailed echocardiographic methods used and distributions of traits in each cohort study are reported in Supplemental Methods and Supplemental Tables 3 and 4.

The present investigation focused on 5 traits of cardiac structure: LVM, LVDD, LVWT, AoD, and left atrial size (LA). Additionally,

we evaluated 2 traits of systolic cardiac function (LVFS and LVSD) and 9 traits of LV diastolic function: Mv-A, Mv-E, E/A, E′, the ratio E/E′ as a surrogate for LV end-diastolic pressure, DecTime, and IVRT, as well as DDpEF and HFpEF (41). Measurements were based on the European and American guidelines for the echocardiographic assessment of the LV (42).

Genotyping methods and imputation

Details on genotyping, imputation, and quality control are presented in Supplemental Table 5. Population stratification as well as family structure, if applicable, was accounted for in each individual cohort's analysis. For replication, 3 of the 6 cohorts (Gutenberg Health Study III [GHS-III]; Cardiovascular Risk Factors, Living and Ageing in Halle [CARLA] study; and Malmö Preventive Project [MPP] study) underwent de novo genotyping using 5′ nuclease assays on 384-well plates. For quality control, genotypes were validated in 10% of the samples for all SNPs.

Definition of traits and statistical methods

Discovery (stage 1). All traits were analyzed as continuous traits, with the exception of LVSD, DDpEF, and HFpEF. LVSD was defined as an EF <50%, fractional shortening (FS) <29% or reduced (poor or impaired) EF by visual estimation. Aggregate binary phenotypes were defined for asymptomatic participants with echocardiographic evidence of LV DDpEF and for those with HFpEF based on information on classes of HF according to the New York Heart Association (NYHA) and medication for HF in addition to echocardiography.

Stage 1 analyses were first performed separately at the individual cohort level for each trait (Figure 1). Continuous echocardiographic traits were related to genotype dosage (0–2 copies of the effect allele) for each autosomal SNP using linear regression assuming additive genetic models adjusted for age, sex, height, weight, and study site (only applicable for the Cardiovascular Health Study [CHS] and Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). For binary traits, we used logistic regression models with the same adjustments. In the Framingham Heart Study (FHS), linear mixed-effects models were applied to account for familial correlations. The associations of genotypes with echocardiographic traits were quantified by beta estimates, SEM, and *P* values. After verifying strand alignment across studies and applying genomic control to each study, we performed inverse variance–weighted fixed-effects meta-analysis across the discovery cohorts with METAL (43) for the structural and the systolic function traits and with the R package MetABEL (http://www.r-project.org) for the diastolic traits. After the meta-analysis, we excluded SNPs with a minor allele frequency (MAF) below 0.5% for diastolic function traits and below 1% for structural traits, and FS and below 3% for LVSD.

We used an a priori *P* value threshold of $<5 \times 10^{-8}$ to indicate genome-wide statistical significance in the discovery meta-analysis for the selection of SNPs taken forward to the next stage. As no SNP reached genome-wide significance in the analysis of diastolic function traits, SNPs with $P < 1 \times 10^{-6}$ were taken forward for replication as "suggestive" findings. This threshold was chosen because there was approximately 80% power to achieve a genome-wide significant *P* value in the combined discovery and replication analysis for most of the traits given the effect sizes observed in the discovery stage. The association results were grouped based on the LD structure from the HapMap (https://www.genome.gov/10001688/international-hapmap-project/) release 28 CEU dataset using PLINK (settings $r^2 > 0.2$, 1 Mb distance) (44). For each identified independent locus, the SNP with the lowest *P* value was defined as the lead SNP and taken forward for replication. SNPs representing loci identified and replicated in our previously published report (2) were not taken forward for replication.

Replication and combined meta-analysis (stage 2). In stage 2, SNPs were related to echocardiographic traits in 6 cohort samples (Figure 1). We chose proxies for 4 of the top SNPs, as no reliable assays were available for wet lab replication of the originally identified SNPs: rs1039692 was used as a proxy for rs949796 (Mv-A, $P = 6.60 \times 10^{-7}$, $R^2 = 1.0$), rs7904368 as a proxy for rs7074647 (E/A, $P = 8.30 \times 10^{-7}$, $R^2 = 0.95$), rs17868167 as a proxy for rs17862703 (IVRT, $P = 9.70 \times 10^{-7}$, $R^2 = 1.0$), and rs806322 as a proxy for rs2762049 (AoD, $P = 3.85 \times 10^{-15}$, $R^2 = 1.0$). The dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/) identifiers of the proxies are reported in the final results.

For the combined meta-analysis of discovery and replication cohorts, SNPs with a *P* value of $<5 \times 10^{-8}$ in the combined meta-analysis were considered to be significantly associated with their respective outcomes, as the overall sample size of the replication cohorts was very small. Genome-wide significant association signals were deemed novel for the corresponding traits if they were >500 kb away from the lead SNPs reported in our previous study (2) and not in high LD with them ($R^2 < 0.5$).

Look-up in other cohorts to test for generalizability of findings

For the genome-wide significant SNPs representing novel loci, we performed "look-ups" in relation to the corresponding echocardiographic traits in children (the Generation R study), Hispanics (NOMAS), and African Americans (meta-analysis of data from JHS and NOMAS). Additionally, we evaluated associations of these SNPs with traits of interest: SNPs for aortic root diameter with pulse wave velocity in the AortaGen consortium (45, 46); and all newly identified SNPs with incident HF and mortality in the CHARGE-HF consortium (3), with all-cause, cardiovascular, and HF mortality in the LURIC study (a cohort of patients with suspected CAD), as well as with MI and CAD in the CARDIOGRAMplusC4D consortium data (47). Further details for the look-up investigations are presented in Supplemental Methods.

Proportion of trait variance explained

The proportion of variance in echocardiographic traits explained by the significantly associated SNPs from our meta-analyses was estimated in 3 of the larger cohorts (Rotterdam study [RS], SHIP, and FHS). Within each cohort, R^2 values of two models were compared for each trait: one model including the covariates (age, sex, height, and weight) only; and one model additionally including the new and known loci. The proportion of the sex-, age-, height-, and weight-adjusted variance explained by all common (MAF >0.01) autosomal genotyped SNPs for each trait was calculated in the SHIP sample using the REML method of GCTA software version 1.24.4 (48).

Known associations of genome-wide significant SNPs

We combined a manual review of the literature with the use of the tool Snipper version 1.2 (http://csg.sph.umich.edu/boehnke/snipper/), which conducts an automated search of the published literature using specified search terms and the putative SNP to evaluate previously reported disease/trait associations for the genome-wide significant SNPs.

cis eQTL analysis

To evaluate the potential functional significance of our findings, we related each replicated SNP to the expression levels of genes in three sets of tissues: human whole blood samples from $n = 5,311$ individuals evaluated by Westra et al. (49), human monocytes from $n = 1,372$ participants in the GHS (50), and LV free-wall tissue from $n = 313$ patients with HF undergoing transplantation and from unused donor hearts from the MAGNet consortium (http://www.med.upenn.edu/magnet). Further details are presented in Supplemental Methods. To evaluate possible *cis* eQTLs across multiple tissues, an additional look-up was performed in the GTEx database for the new findings.

Pathway analysis

The collective effects of multiple genetic variants on biological systems were investigated by pathway analysis, first for the 7 structural and systolic traits combined, and then for the 9 combined diastolic traits and for all 16 echocardiographic traits combined (for details, see Supplemental Methods).

To identify whether any of the associated SNPs fall within regulatory regions of the genome, we evaluated data from ENCODE (17). We compared the expected overlap of the putative SNPs with functional domains due to chance with the actual observed overlap by creating a permuted set of non-associated SNPs that were evaluated for overlap with the functional domains. We also used the DEPICT tool to further explore functionality of the identified SNPs (19). In addition, variants with $P < 5 \times 10^{-7}$ were used as the input for the DAPPLE software (18), which then built both direct and indirect interaction networks from seed genes near the top loci.

Statistics

If not specified otherwise, a Wald test statistic was calculated by dividing the estimated effect size by its standard error and comparing them with a normal distribution (2-tailed) with mean zero. In the GWAS, $P < 5 \times 10^{-8}$ for the combined stage 1 and 2 analysis was deemed significant (11), which corresponds to a significance level of 0.05 after correcting for 1 million independent SNPs (51). For pathway analyses, a FDR was applied as multiple testing correction with a cutoff-value <0.05 for statistical significance.

Study approval

All study protocols of participating cohorts were reviewed and approved by a local ethics committee and followed the recommendations of the Declaration of Helsinki. All subjects in the cohorts provided informed written consent prior to their participation in the study. Therefore, no specific approval was required for this meta-analysis of human data. The institutional review boards are listed in the supplemental material.

Author contributions

Conception and study design: AH, A. Peters, AS, AZ, BMP, BP, DT, EB, EI, GFM, H. Schmidt, H. Schunkert, JFF, LJL, LK, MD, MF, MK, OTR, PPP, PSW, R. Schmidt, RZ, SB, SBF, SJS, TBH, TL, TM, TZ, RSV, V. Gudnason, and WM. Sample collection and phenotyping: ADH, AJB, A. Peters, APM, ASPS, A.V. Smith, A.V. Stanton, BMP, CML, DCS, DKA, D. Mascalzoni, DR, DT, EB, EI, EJB, ERF, FE, FN, GFM, H. Schunkert, HV, JA, JM, JS, JWD, KJL, KL, LJL, L. Lannfelt, L. Lind, MC, MD, MGL, MJGL, MK, ML, MR, MRDT, NF, NLS, OTR, PMN, PS, PSW, RL, RLS, RM, R. Schmidt, R. Sorice, RSV, RW, RZ, SB, SC, SJS, SKM, S. Nappo, ST, STR, TBH, TES, TL, TM, TN, TZ, V. Großmann, and V. Gudnason. Genotyping: AAH, AGU, AMT, A. Pfeufer, APM, CML, DR, EI, ERF, FR, H. Schmidt, JGS, JH, JIR, JS, KJL, KL, L. Lind, LPL, MC, MEK, MF, MM, MRI, OM, PSW, R. Sorice, SB, SKM, TES, TL, TN, TPC, TZ, UB, UV, and V. Großmann. Statistical analysis: AS, AT, CL, C. Minelli, C. Monnereau, C. Müller, DCS, D. Malzahn, D. Medenwald, DP, DR, DT, EH, FDGM, GFM, HL, JAB, JCB, JFF, JGS, JK, JNH, JS, KMR, LF, LPL, MC, MEK, MF, MGL, MHC, MM, MMN, MRI, NDD, PS, PSW, R. Sorice, SJS, SKM, S. Nuding, SP, and THP. Interpretation of data: ACM, ADH, AGU, AH, AJB, AS, ASPS, AT, A.V. Smith, A.V. Stanton, AZ, BHS, BP, CAM, DKA, EB, FR, GFM, HL, H. Schmidt, H. Schunkert, JFF, JGS, JM, JNH, JWD, KL, LF, LJL, MD, MF, MGL, MJGL, ML, MM, NS, OHF, OM, PMN, PS, PSW, R. Schmidt, RSV, SBF, SJS, ST, TBH, THP, TPC, TZ, V. Gudnason, VWVJ, WL, and WM. Drafting the manuscript: AS, AT, JFF, MD, PSW, RSV, and TZ. Revising manuscript critically for important intellectual content: all authors.

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